REMARKS

The non-elected claims 3-20, 22 and 24 and claim 23 have been canceled without prejudice. Claims 1 and 21 have been amended. Claims 1, 2 and 21 are pending and at issue.

Claim 1 and 21 have been amended to replace the term "insect" in step (i) with "*Tenebrio molitor* or *Holotrichia diomphalia*". Support for this amendment can be found in Examples 1 and 4 of the specification.

No new matter has been introduced by these amendments. Entry and consideration of the amendments is therefore respectfully submitted.

Rejections Under 35 U.S.C. § 102(b), Should Be Withdrawn

Leonard Reference:

Claims 1, 2, 21 and 23 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Leonard et al. (Insect Biochem, Vol. 15, No. 6, pp. 803-810).

Claim 23 has been canceled without prejudice.

Leonard discloses the presence of phenoloxidase (PO) in both plasma and haemocyte fractions separately. Leonard also specifically teaches that use of an anti-coagulant is necessary for determining PO activity in plasma, but then finds that plasma contains less than 50% of the PO activity of the haemocyte fractions after activation due to the presence of the anti-coagulant. Leonard thus concludes that the "haemocytes are the main repository for proPO" (see page 805, col. 2, first paragraph under "Results"). Leonard makes **no mention** of a composition of combined plasma and haemocyte fractions that enables the use of detection of β-1,3 glucans..

Since Leonard makes no mention of a composition of combined plasma and haemocyte fractions, and further, explicitly **discourages** using plasma to determine PO activity due to the inactivation of PO by anti-coagulants, Leonard cannot anticipate the present claims. The present claims are directed to a composition derived from a combination of plasma and haemocyte fractions for detecting the presence of a PO activator, i.e., β-1,3, glycan. By contrast, Leonard

teaches that while β-1,3, glycan can be used as a PO activator to determine the presence of PO (and not the contrary, as recited in the present claims), Leonard achieves this using haemocyte fractions **only**, not plasma, much less a combination. Accordingly, Leonard cannot anticipate the present claims.

In addition, Leonard discloses fractions obtained from haemocyte lysate of *Blaberus craniifer* exhibiting phenoloxidase activity (see, Table 1 of the Leonard reference). Leonard does not disclose a mixture of plasma and hemocyte lysate from *Tenebrio molitor* or *Holotrichia diomphalia* exhibiting phenoloxidase activity as called for in claim 1. Claim 21 calls for lysate treated *Tenebrio molitor* or *Holotrichia diomphalia* plasma fractions exhibiting phenoloxidase activity. Leonard does not disclose a composition comprising lysate treated *Tenebrio molitor* or *Holotrichia diomphalia* plasma fractions. Accordingly, the subject matter of claims 1, 2 and 21 of the present application is novel over Leonard.

Asokan Reference:

Claims 1 and 21 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Asokan et al. (Dev. And Comp. Immun., Vol. 21, No. 1, pp 1-12).

Asokan discloses PO activity in plasma and haemocytes of the marine mussel *Perna viridis* (see Abstract of the Asokan reference). Asokan also discloses prophenoloxidase (proPO) enzyme in insect and crustacean haemocytes, either in *both* plasma and haemocyte fractions (see page 2, column 1 of the Asokan reference).

There are several differences between the presently claimed invention and Asokan. First, Asokan also teaches that more than **20 times** the amount of proPO is found in haemocyte fractions than in plasma, indicating its *intracellular* presence in haemocytes. Despite this, Asokan cautions the use of anticoagulant in studies involving PO assays in fractionating haemolymph (which includes plasma and haemocytes) since it can destroy up to 50% of the PO activity even after removal of the coagulant (page 2, col. 1, second paragraph). Asokan recommends that the ideal way to determine the distribution of PO in cell-free plasma and cellular haemocyte fractions would be to use an invertebrate whose blood does not coagulate *in vitro*, and further describes such an

invertebrate (*Perna viridis*). Thus, Asokan determines the presence of PO in *both* haemocyte and plasma fractions of *Perna viridis*, separately. However, it remains that Asokan does not teach or disclose *combining* the two untreated fractions as recited in the present claims, much less to determine the presence of β-1,3 glucans.

Second, Asokan teaches measuring PO activity in plasma and haemocyte fractions by adding β-1,3-glucans (and bacterial LPS). To the contrary the present invention discloses the converse, i.e., identification of β-1,3-glucans by determining if combined fractions or plasma and haemocytes exhibit PO activity. While Asokan's primary objective was to activate proPO by the addition of activators, the present claims depend on the proPO already having been activated to PO by the presence of an activator, which indicates the presence of a pathogen. Asokan distinguishes PO and proPO as stated below:

> "In arthropods, PO exists in haemolymph as an inactive enzyme, prophenoloxidase (proPO), which is activated to PO by both an endogenous activating system and exogenous reagents such as lipids, detergents and organic solvents (4-7)." See page 1, column 2 of the Asokan reference.

Thus, Asokan only discloses the presence of the unactivated proPO in insect and crustacean plasma and haemocyte fractions (as indicated by its conversion to PO upon activation). In addition, as described above, Asokan fails to describe a **composition** comprising combined plasma and haemocyte fractions which **already** exhibits PO activity (i.e., is already activated due to the presence of β-1,3 glycans), as called for in the claimed invention. In fact, Asokan's disclosure is mutually exclusive with the present claims and defeats the purpose of the present claims, since according to Asokan, β-1,3 glycans (or LPS) **must be added** in order for the plasma and haemocyte fractions to exhibit PO activity, while the present claims recite a composition isolated from the combination of plasma and haemocyte fractions that already exhibit PO activity. At best, Asokan describes the presence of proPO in insect and crustacean plasma and haemocyte fractions, but not the presence a composition comprising β-1,3-glucan (due to the presence of already-activated proPO).

Third, the present claims have been amended to recite that the combined plasma and haemocyte fractions are from *Tenebria molitor* and *Holotrichia dimophalia*. *Tenebria molitor* and *Holotrichia dimophalia* exhibit surprisingly high phenoloxidase levels compared to other insects, i.e., *Drosophila melangaster* and *Galleria mellonella* upon treatment with β-1,3-glucan. See Exhibit A, red bars. This result may be due to *Tenebria molitor* and *Holotrichia dimophalia*'s relatively large larva size compared to other insects. The larger size larva enables easier and larger-scale extraction of hemoloymph and as a result increased PO activity.

This result may additionally be attributed to the fact that the initially collected hemolymph of *Tenebrio molitor* and *Holotrichia diomphalia* show lower PO activity compared to hemolymph from other insects, i.e., *Drosphila melangaster* and *Galleria mellonella*. See Exhibit A, yellow bars. This initial sample from *Tenebrio molitor* and *Holotrichia diomphlia* contains proPO which is activated to phenoloxidase upon treatment with β-1,3-glucan. It is advantageous to have low po activity-which indicates that the enzyme is in its inactive form (proPO)-prior to treatment with β-1,3-glucan.

Therefore, since Asokan clearly does not disclose a composition comprising a mixture of plasma and haemocyte lysate or a lysate treated plasma from *Tenebrio molitor* or *Holotrichia diomphalia* as discussed above.

As the Examiner is well aware, in order to anticipate a claim under 35 U.S.C. 102(b), a single prior art reference must disclose each and every claim limitation. Asokan does not disclose each and every limitation of present claims 1 and 21.

For the foregoing reasons, claims 1 and 21 are not disclosed by Asokan and withdrawal of this ground for rejection is believed to be in order.

The Examiner quires about claim 23 regarding how it further limits claim 2 from which it depends. Claim 23 has been canceled without prejudice.

Rejections Under 35 U.S.C. § 103(a), Should Be Withdrawn

Claims 2 and 23 are rejected under 35 U.S.C. § 103(a) as obvious over the combination of Asokan in view of Ashida.

Claim 23 has been canceled without prejudice.

The Examiner alleges that the present claim 2, which recites that the combination of haemocyte and plasma franctions is useful for detecting glucans at concentrations as low as 20 pg/ml, reads on the concentrations disclosed in the Ashida reference (higher than 0.1 ng/ml). The Examiner contends that Asokan teaches every other limitation in claims 2 and 23 except for the 20 pg/ml concentration.

First, Applicants respectfully assert that there would have been no motivation to combine Asokan and Ahsida for the following reasons. The differences between the present claims and the Asokan reference has been discussed, *supra*, in connection with the rejections over 35 U.S.C. § 102(b). Asokan discloses PO activity in plasma and haemocytes of the marine mussel *Perna viridis* and proPO in insect and crustacean haemocytes, in both haemocyte and plasma fractions. Asokan does not teach or suggest a composition comprising a **mixture** of plasma and hemocyte lysate (much less from *Tenebrio molitor* or *Holotrichia diomphalia*) exhibiting PO activity. Moreover, Asokan does not teach or suggest the detection sensitivity of the presently claimed fractions.

To the contrary, Asokan teaches that, while PO activity is in both plasma and haemocyte fractions, 20 times *more* is found in haeomcyte fractions (i.e., intracellularly in haemocytes) than the plasma (extracellularly). This actually teaches away from the method of the present invention, which discloses measuring PO activity in the *combined* hemolymph and PO fractions.

Ashida does not overcome the deficiencies of Ashida. Ashida teaches away from measuring PO activity entirely, due to the problems of self-oxidation of PO, an unstable enzyme, and unattainable markedly enhanced detection sensitivity. To the contrary, Ashida teaches determining the presence of β-1,3 glycan by measuring the activity of **another enzyme** altogether (PPAE). Further, the compositions of Ashida have limited sensitivity and can only detect β-1,3-

glucan concentrations higher than 0.1 **ng**/ml (nanograms are 10^{-9}) (see Fig. 3 on sheet 2 of 4 of Ashida). By contrast, the composition of claim 2 can detect β-1,3-glucan concentrations as low as 20 **pg**/ml (picograms are 10^{-12}). This is a very significant difference.

Thus, there would have been no motivation to combine the Asokan and Ashida references since both teach away from the present claims.

In addition, the Examiner must consider the **unexpected results** of the present claims with respect to the low-threshold detection sensitivity. Such sensitivity would not be expected since the references teach that plasma has much lower activity due to the necessary addition of anti-coagulant.

This was surprising and unexpected for the following reasons. First, the present application itself teaches that the PO composition derived from plasma **did not correlate** with the β-1,3, glycan concentration (Comparative Example 1). This is contrasted with the teachings of Example 1, where the **mixture** of the plasma and haemocyte fractions detected β-1,3-glycan presence as a function of its concentration (Figure 1), but neither the haemocyte fraction nor the plasma alone could be used to detect the presence of β-1,3, glycan at this low concentration.

Similarly, Example 4 and Figure 7 of the present specification show that even when separated and individually purified, *then* combined, the resulting combination of plasma and haemocyte fractions **has PO activity** (thus enabling detection of the presence of low β-1,3, glycan concentrations) wherein each of the individual compositions do not.

Accordingly in view of the teaching away by the references of using plasma in the first place, and the teachings in the specification that only the combination worked, there could have been no motivation to combine, much less a reasonable expectation of success, or the super-sensitive capacity ultimately achieved by the present invention. The Examiner is respectfully requested to consider the Federal Circuit's decision in *Richardson-Vicks Inc. v. Upjohn Co.,* 122 F.3d 1476 (Fed. Cir. 1997), which held that "primary" among evidence of non-obviousness is the secondary consideration of unexpected synergy between two agents (in that case, ibuprofen and the pseudoephedrine).

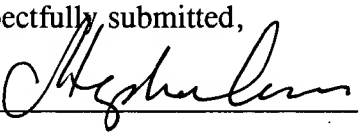
It is respectfully submitted that the present invention has superior sensitivity for detecting β-1,3-glucan. This is surprising and entirely unexpected. A skilled person could not have expected these improved properties without the benefit of the teachings in the present application, even upon considering the combined teachings of Asokan and Ashida. For this reason, and the other reasons discussed above, the present claims are not obvious over Asokan in view of Ashida and this rejection should be withdrawn.

Conclusion

In light of the above mentioned amendments and arguments, all of the pending claims in this application are believed to be in condition for allowance. Entry and consideration of these amendments and remarks are therefore respectfully requested. The Examiner is invited to contact Applicants' representative at the below-indicated telephone number if he believes it would advance prosecution of the application. An allowance is earnestly sought.

Dated: July 28, 2004

Respectfully submitted,

By 

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